



**UNIVERSITI PUTRA MALAYSIA**

***Andrographis paniculata* Nees AND *Orthosiphon stamineus* Benth  
GROUND LEAF AS ANTIBIOTIC AND ANTIOXIDANT SUPPLEMENTS  
FOR BROILER CHICKEN**

**MASNINDAH BINTI MALAHUBBAN**

**ITA 2014 3**



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FOR BROILER CHICKEN**



By

**MASNINDAH BINTI MALAHUBBAN**

**Thesis Submitted to the School of Graduate Studies, Universiti Putra  
Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of  
Philosophy**

**September 2014**

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirements for the degree of Doctor of Philosophy

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**September 2014**

**Chairman: Professor Abd. Razak Bin Alimon, Ph.D.**

**Faculty: Institute of Tropical Agriculture**

The use of synthetic antibiotics and antioxidants has creates serious obstacles and hazards, to farmers, consumers and the environment. Antibiotics and antioxidant from natural sources can potentially be useful to overcome these problems. Therefore, present study was conducted to evaluate the selected medicinal plants, *Andrographis paniculata* and *Orthosiphon stamineus* for antibiotics and antioxidant potential and subsequently to evaluate the broiler performance by inclusion the *A. paniculata* and *O. stamineus* ground leaf in diets.

Initially, *A. paniculata* and *O. stamineus* ground leaf were evaluated for their *in vitro* antibiotic and antioxidant potential by extracting the ground leaf in four solvents, namely water, methanol, ethanol and chloroform and subsequently testing using the disc diffusion method for antibiotic potential and selected radical scavenging activities for antioxidant potential. Qualitative screening for bioactive compound on both extracts by using methanol as solvent has indicated the presence of alkaloid, saponin, flavonoid, tannin, terpenoid and steroid. High performance liquid chromatography analysis indicated that andrographolide and rosmarinic acid were the major compound from *A. paniculata* and *O. stamineus*, respectively. It was found that the highest yields of these two compounds were obtained by methanol extraction and that substantial antibiotic and antioxidant properties were exhibited by these compounds. In addition, it was showed that the *O. stamineus* extract contained higher antioxidant capacity than *A. paniculata* extract.

The ground leaf were incorporated in broiler diets at levels of 0, 2, 4, and 8 g/kg and the influence on growth performance, carcass characteristics, serum biochemistry, and intestinal and liver morphology was examined in a 42-d feeding trial. It was involved 280 one-day old male broiler chickens, grown, maintained and received *ad libitum* water and diet. The feeding trial was started from 21 days old and assigned with respective treatments. At day-42, the broiler chickens were slaughtered and analysed and it was found that broilers fed *O. stamineus* ground leaf at a rate 8 g/kg was the most promising dietary supplement to promote overall growth performance without deleterious effects on carcass characteristics, serum biochemical properties and morphological components of liver and intestine compared with *A. paniculata* ground leaf and control diets. In addition to promoting weight gain, it reduced abdominal fat and serum cholesterol. It also maintained the integrity of liver, thus indicating that no toxic effect from *O. stamineus* supplementation at a rate up to 0.8 g/kg. Besides that, 8 g/kg *O. stamineus* supplementation improved intestinal structure, especially in the duodenum. Present study also found that the inclusion of *O. stamineus* ground leaf at 8 g/kg in the broiler diet increased total tract N retention and apparent metabolizable energy.

Based on these results, *O. stamineus* ground leaf at 8 g/kg was selected for the next *in vivo* experiment. Present study was conducted to evaluate the response of broiler due to antibiotic and antioxidant properties of *O. stamineus* by comparing its potential with tetracycline and Vitamin E supplementation in diets, as positive controls. The study was involved 160 of a one-day old male broiler chickens, and had *ad libitum* water and feed for up to 20-day old. The respective treatments were assigned and initiated at 21-day old male broiler chickens. After slaughtering, data were recorded and analysed at 42-day old male broiler chickens, and it was found that *O. stamineus* ground leaf supplement at a rate of 8 g/kg in broiler diet results in growth performance similar to that of tetracycline and Vitamin E supplementation. In addition, it was also found that 8 g/kg *O. stamineus* supplementation in diet promoted serum enzymes-lowering effect. In contrast, high serum enzymes activity showed in broiler fed tetracycline supplement. The inclusion of 8 g/kg *O. stamineus* ground leaf in diet of broiler enhanced meat quality by stabilizing sensory properties, meat colour and meat pH. The results also indicated that the inclusion of *O. stamineus* leaf ground at 8 g/kg in diet was comparable with 200 mg/kg Vitamin E supplementation in diet. It was found that all dietary treatments maintained intestinal population of *Lactobacillus* and *Escherichia coli*. However, the inclusion of 8 g/kg *O. stamineus* or tetracycline in diet inhibited the population of facultative anaerobe. Therefore, the use of *O. stamineus* ground leaf as supplement in broiler chicken diet has the potential to promote and maintain growth and gut health and subsequently creates safe and sustainable broiler chicken production.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah.

**SERBUK DAUN *Andrographis paniculata* Nees DAN *Orthosiphon stamineus* Benth SEBAGAI ADITIF ANTIBIOTIK DAN ANTIOKSIDAN UNTUK AYAM PEDAGING**

Oleh

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Penggunaan antibiotik dan antioksidan sintetik telah menyebabkan masalah dan bencana yang serius kepada petani, pengguna dan persekitaran. Antibiotik dan antioksidan dari sumber semulajadi dilihat berpotensi untuk digunakan bagi mengatasi masalah ini. Maka kajian ini dijalankan untuk menilai tumbuhan perubatan yang terpilih iaitu *Andrographis paniculata* dan *Orthosiphon stamineus* yang berpotensi sebagai sumber antibiotik dan antioksidan, seterusnya kajian ini turut menilai prestasi ayam pedaging kesan dari pengambilan makanan yang mengandungi serbuk daun *A. paniculata* dan *O. stamineus*.

Kajian dimulakan dengan menilai potensi antibiotik dan antioksidan serbuk daun *A. paniculata* dan *O. stamineus* secara *in vitro* dengan mengekstrak serbuk daun tersebut dalam empat jenis bahan pelarut iaitu air, methanol, etanol dan klorofom, dan seterusnya ia diuji menggunakan kaedah sebaran cakera bagi menentukan potensi antibiotik dan aktiviti-aktiviti pencarian radikal bagi penentuan potensi antioksidan. Saringan kualitatif telah dijalankan, dan ia menunjukkan kedua-dua serbuk daun yang diekstrak menggunakan metanol mengandungi alkaloid, saponin, flavonoid, tannin, terpenoid dan steroid. Analisis kromatografi cecair berprestasi tinggi menunjukkan andrographolida dan asid rosmarinik merupakan kompaun utama masing-masing dari *A. paniculata* dan *O. stamineus*. Kajian mendapati hasilan paling tinggi bagi kedua-dua kompaun terbabit diperolehi daripada pengekstrakan methanol dan ciri-ciri antibiotik dan antioksidan yang menggalakkan ditunjukkan oleh kedua-dua kompaun terbabit.

Serbuk daun berkenaan dicampurkan ke dalam diet ayam pedaging pada beberapa paras kandungan iaitu 0, 2, 4, dan 8 g/kg bagi menentukan pengaruhnya ke atas prestasi pertumbuhan, ciri-ciri karkas, biokimia serum,

dan morfologi usus dan hati, dan kajian cubaan pemakanan ini dilakukan sehingga ayam pedaging berusia 42 hari. Kajian ini melibatkan ayam pedaging jantan yang diperolehi pada ketika usianya satu hari, dipelihara dan dijaga. Cubaan pemakanan dimulakan ketika ayam pedaging berusia 21 hari dan seterusnya disusun mengikut rawatan tersebut. Pada usianya 42 hari, ayam pedaging disembelih dan dianalisis, dan keputusan mendapati ayam pedaging yang makan 8 g/kg serbuk daun *O. stamineus* merupakan makanan tambahan yang paling berpotensi untuk merangsangkan prestasi pertumbuhan tanpa kesan-kesan negative ke atas ciri-ciri karkas, kandungan biokimia serum dan komponen morfologi hati dan usus berbanding dengan serbuk daun *A. paniculata* dan diet-diet kawalan. Tambahan kepada penggalakan peningkatan berat, ia telah mengurangkan lemak perut dan kolesterol serum. Ia juga memelihara integriti hati, dan hal ini menunjukkan tiadanya kesan toksik dari *O. stamineus* yang ditambah di dalam pemakanan sebanyak 8 g/kg. Di samping itu, penambahan 8 g/kg *O. stamineus* memperbaiki struktur usus, terutamanya duodenum. Kajian ini turut mendapati penambahan 8 g/kg serbuk daun *O. stamineus* meningkatkan jumlah zon pengumpulan N dan tenaga sebenar yang boleh dimetabolikkan.

Berdasarkan kepada keputusan tersebut, 8 g/kg serbuk daun *O. stamineus* telah dipilih untuk eksperimen *in vivo*. Kajian ini dijalankan untuk menilai tindakbalas ayam pedaging kesan dari kandungan antibiotik dan antioksidan *O. stamineus* dengan membandingkannya dengan penambahan tetrasiklin dan Vitamin E di dalam pemakanan, sebagai kawalan positif. Kajian ini melibatkan 160 ekor ayam pedaging jantan yang dipelihara sejak berusia satu hari. Rawatan kajian dimulakan semasa ayam pedaging berusia 21 hari. Data mula direkodkan dan dianalisis ketika berusia 42 hari. Kajian menunjukkan serbuk daun *O. stamineus* pada kadar 8 g/kg mempunyai prestasi pertumbuhan yang menyamai prestasi tetrasiklin dan Vitamin E. Tambahan lagi, ia juga didapati mampu merangsang kesan kekurangan enzim serum. Sebaliknya, aktiviti enzim serum yang tinggi didapati pada ayam pedaging yang dirawat dengan pemakanan tambahan yang mengandungi tetrasiklin. Penambahan 8 g/kg serbuk daun *O. stamineus* di dalam makanan ayam pedaging telah meningkatkan kualiti daging dengan menstabilkan komponen rasa, warna dan pH daging. Kajian turut menunjukkan ia setanding dengan penambahan sebanyak 200 mg/kg Vitamin E. Selain itu, rawatan tambahan pemakanan ini memelihara populasi *Lactobacillus* dan *Eschericia coli* di dalam usus. Walau bagaimanapun, penambahan 8 g/kg serbuk daun *O. stamineus* merencatkan populasi fakultatif anaerob. Dengan ini, penggunaan serbuk daun *O. stamineus* sebagai tambahan di dalam pemakanan ayam pedaging berpotensi untuk merangsang dan mengekalkan pertumbuhan dan kesihatan perut ayam pedaging dan seterusnya mewujudkan pengeluaran ayam pedaging yang selamat danlestari.



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I certify that a Thesis Examination Committee has met on (date of viva) to conduct the final examination of Masnindah Binti Malahubban on her thesis entitled “***Andrographis paniculata* Nees AND *Orthosiphon stamineus* Benth Ground Leaf as Antibiotic and Antioxidant Supplements for Broiler Chicken**” in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U. (A) 106] 15 March 1998. The Committee recommends that the students be awarded the Doctor of Philosophy.

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## TABLE OF CONTENTS

	Page
ABSTRACT	i
ABSTRAK	iii
ACKNOWLEDGEMENTS	v
APPROVAL	vi
DECLARATION	viii
LIST OF TABLES	xiv
LIST OF FIGURES	xvi
LIST OF ABBREVIATIONS	xviii
<b>CHAPTER</b>	
<b>1</b>	<b>GENERAL INTRODUCTION</b> <span style="float: right;"><b>1</b></span>
<b>2</b>	<b>LITERATURE REVIEW</b> <span style="float: right;"><b>3</b></span>
	2.1 Broiler Production and their Obstacles <span style="float: right;">3</span>
	2.2 Mechanism and Application of Antibiotics <span style="float: right;">5</span>
	2.3 Mechanism and Application of Antioxidants <span style="float: right;">7</span>
	2.4 Natural Antibiotics and Antioxidants Actions <span style="float: right;">10</span>
	Associated with Animals
	2.5 Application of Herbal Feed Additives in Poultry <span style="float: right;">12</span>
	Production
	2.6 Potential of Selected Medicinal Herbs, <span style="float: right;">14</span>
	<i>Andrographis paniculata</i> Nees and <i>Orthosiphon</i>
	<i>stamineus</i> Benth Supplementation in Animal diet
	2.6.1 <i>Andrographis paniculata</i> : Botanical, <span style="float: right;">14</span>
	Chemical Characteristics, and Uses
	2.6.2 <i>Orthosiphon stamineus</i> : Botanical, <span style="float: right;">17</span>
	Chemical Characteristics, and Uses
<b>3</b>	<b>PHYTOCHEMICAL ANALYSIS OF <i>Andrographis</i></b> <span style="float: right;"><b>20</b></span>
	<b><i>paniculata</i> AND <i>Orthosiphon stamineus</i> LEAF</b>
	<b>EXTRACTS FOR THEIR ANTIBACTERIAL AND</b>
	<b>ANTIOXIDANT POTENTIAL</b>
	3.1 Introduction <span style="float: right;">20</span>
	3.2 Materials and Methods <span style="float: right;">21</span>
	3.2.1 Chemicals <span style="float: right;">21</span>
	3.2.2 Plant Materials <span style="float: right;">21</span>
	3.2.3 Sample Preparation <span style="float: right;">22</span>
	3.2.4 Qualitative Screening of Bioactive <span style="float: right;">22</span>
	Compounds
	3.2.4.1 Alkaloid Test <span style="float: right;">22</span>
	3.2.4.2 Saponin Test <span style="float: right;">22</span>
	3.2.4.3 Flavanoids Test <span style="float: right;">22</span>
	3.2.4.4 Tannins Test <span style="float: right;">22</span>
	3.2.4.5 Steroids and Terpenoids Test <span style="float: right;">23</span>
	3.2.5 Quantification of major active compound <span style="float: right;">23</span>

	in <i>A. paniculata</i> and <i>O. stamineus</i> by using High Performance Liquid Chromatography (HPLC) Analyses.	
	3.2.5.1 Preparation of Sample and Standard Solution	23
	3.2.5.2 Chromatographic Conditions	23
	3.2.6 Antibacterial Potential of <i>Andrographis paniculata</i> and <i>Orthosiphon stamineus</i> Leaf Extracts Prepared with Different Solvents	24
	3.2.6.1 Test Bacteria	24
	3.2.6.2 Disc Diffusion Assay	24
	3.2.7 Antioxidant Potential of <i>Andrographis paniculata</i> and <i>Orthosiphon stamineus</i> Leaf Extracts Prepared with Different Solvents	25
	3.2.7.1 Determination of Total Phenolic Content	25
	3.2.7.2 Determination of Free Radical-Scavenging Activity	25
	3.2.7.3 Determination of Superoxide Radical-Scavenging Activity	26
	3.2.8 Statistical Analyses	26
	3.3 Results	27
	3.3.1 Qualitative Screening for Bioactive Compounds	27
	3.3.2 Analysis of Andrographolide in <i>A. paniculata</i> Leaf Extracts by HPLC	28
	3.3.3 Analysis of Rosmarinic Acid in <i>O. stamineus</i> Leaf Extracts by HPLC	30
	3.3.4 Antibacterial Activity of <i>A. paniculata</i> and <i>O. stamineus</i> Leaf Extracts Prepared with Different Solvents	33
	3.3.5 Antioxidant Activity of <i>A. paniculata</i> and <i>O. stamineus</i> Leaf Extracts Prepared with Different Solvents	35
	3.3.5.1 Total Phenolic Content	35
	3.3.5.2 DPPH and Superoxide Scavenging Activities	36
	3.4 Discussion	38
	3.5 Conclusion	40
<b>4</b>	<b>GROWTH PERFORMANCE, CARCASS CHARACTERISTICS, SERUM BIOCHEMISTRY, LIVER AND INTESTINE MORPHOLOGY OF BROILERS FED <i>Andrographis paniculata</i> AND <i>Orthosiphon stamineus</i> GROUND LEAF DIETARY SUPPLEMENTS</b>	<b>41</b>
	4.1 Introduction	41
	4.2 Materials and Methods	42

4.2.1 Birds and Experimental Design	42
4.2.2 Preparation of Herbs for Animal Feed Additive	44
4.2.3 Proximate Analysis	45
4.2.3.1 Determination of Moisture and Dry Matter	45
4.2.3.2 Determination of Ash	45
4.2.3.3 Determination of Crude Protein	45
4.2.3.4 Determination of Fat	46
4.2.3.5 Determination of Energy	47
4.2.4 Performance Indices Measurement	48
4.2.4.1 Average Daily Gain (ADG)	48
4.2.4.2 Feed Conversion Ratio (FCR)	48
4.2.4.3 Mortality Rate	48
4.2.5 Carcass Characteristics	48
4.2.6 Digestibility Trial	49
4.2.7 Blood Collection	49
4.2.8 Histological Procedures	49
4.2.8.1 Tissue Preservation and Processing	50
4.2.8.2 Staining Procedures	50
4.2.8.3 Gastrointestinal Tract Examination	50
4.2.8.4 Liver Examination	51
4.2.8 Statistical Analyses	51
4.3 Results	51
4.3.1 Live Weight and Weight Gain	51
4.3.2 Feed Intake, Feed Conversion Ratio and Mortality	54
4.3.3 Carcass Characteristics and Organs	54
4.3.4 Digestibility of Dry Matter, Crude Protein and Apparent Metabolizable Energy of Diets	58
4.3.5 Serum Biochemistry	58
4.3.6 Morphological Analysis of Intestine and Liver	64
4.4 Discussion	68
4.5 Conclusion	72

**5 THE EFFECT OF DIETARY SUPPLEMENTATION OF *Orthosiphon stamineus* GROUND LEAF ON GROWTH PERFORMANCE, MEAT CHARACTERISTICS, INTESTINAL MICROFLORA AND LIPID PEROXIDATION IN BROILER CHICKENS** 73

5.1 Introduction	73
5.2 Materials and Methods	74
5.2.1 Birds and Experimental Design	74
5.2.2 Preparation of <i>Orthosiphon stamineus</i> used as An Animal Feed Additive	76



5.2.3	Measurement of Performance Indices	76
5.2.4	Blood collection	76
5.2.5	Intestinal Bacteria Count	77
5.2.6	Broiler Meat Characteristics	77
5.2.6.1	Water Holding Capacity	77
5.2.6.2	Proximate Composition	77
5.2.6.2.1	Total Moisture	77
5.2.6.2.2	Total Fat	78
5.2.6.2.3	Total Protein	78
5.2.6.3	Sensory Evaluation	78
5.2.6.4	Meat Colour	78
5.2.6.5	Meat pH Measurement	78
5.2.6.6	Measurement of Antioxidative Potential	79
5.2.7	Statistical Analysis	81
5.3	Results	82
5.3.1	Broiler Weight Performance	82
5.3.2	Feed Intake, Feed Conversion Ratio and Mortality	83
5.3.3	Carcass and Organ Characteristics	83
5.3.4	Serum Biochemistry	84
5.3.5	Lipid Peroxidation in Serum and Liver	86
5.3.6	Intestinal Microbial Population	87
5.3.7	Meat Characteristics	88
5.3.7.1	Water Holding Capacity, Total Phenolics and Proximate Analysis	88
5.3.7.2	Sensory Evaluation	89
5.3.7.3	Meat Colour	89
5.3.7.4	Meat pH	91
5.3.7.5	Anti-oxidative Activity of Meat	92
5.4	Discussion	93
5.5	Conclusion	97
<b>6</b>	<b>GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE RESEARCH</b>	<b>98</b>
	<b>REFERENCES</b>	<b>102</b>
	<b>APPENDICES</b>	<b>118</b>
	<b>BIODATA OF STUDENT</b>	<b>128</b>
	<b>LIST OF PUBLICATIONS</b>	<b>129</b>

## LIST OF TABLES

Table		Page
2.1	Top ten chicken meat producers in Asia in 2010 ('000 tonnes).	3
2.2	Critical differences between antibiotic and herbal plant based products.	7
2.3	Antioxidants and mechanism of action.	8
2.4	Types of oxidation inhibitors (antioxidants).	9
2.5	Herbal plants and their responses in poultry.	13
3.1	Qualitative analysis of bioactive compounds in <i>A. paniculata</i> and <i>O. stamineus</i> leaf extracts prepared in different solvents.	27
3.2	HPLC quantification of andrographolide in <i>A. paniculata</i> leaf extract and rosmarinic acid in <i>O. stamineus</i> leaf extract.	30
4.1	Ingredients in the basal diet and nutritional analysis.	43
4.2	Weekly live body weight as of day-21 to day-42 of broilers fed on diets supplemented with different rates of <i>A. paniculata</i> and <i>O. stamineus</i> ground leaf (g $\pm$ SE).	52
4.3	Weekly weight gain as of week-4 to week-6 of broilers fed on diets supplemented with different rates of <i>A. paniculata</i> and <i>O. stamineus</i> ground leaf (g $\pm$ SE).	53
4.4	Weekly feed intake, feed conversion ratio (FCR) and mortality as of week-4 to week-6 of broilers fed on diets supplemented with different rates of <i>A. paniculata</i> and <i>O. stamineus</i> ground leaf (Mean $\pm$ SE).	55
4.5	Carcass characteristics as of day-42 of broilers fed on diets supplemented with different rates of <i>A. paniculata</i> and <i>O. stamineus</i> ground leaf (Mean $\pm$ SE).	56
4.6	Organs weight expressed as percentage of live-weight as of day-42 of broilers fed on diets supplemented with different rates of <i>A. paniculata</i> and <i>O. stamineus</i> ground leaf (Mean $\pm$ SE).	57
4.7	Digestibility of dry matter, crude protein and apparent metabolizable energy of the experimental diets (Mean $\pm$ SE).	59
4.8	Serum biochemical parameters of broilers at initial (day-21) and at the end (day-42) of dietary treatments (Mean $\pm$ SE).	61

4.9	Sodium, potassium, chlorine and urea levels in serum of broiler fed on diets supplemented with different rates of <i>A. paniculata</i> and <i>O. stamineus</i> ground leaf (Mean $\pm$ SE).	62
4.10	Serum enzymes in broiler fed different dietary treatments (Mean $\pm$ SE).	63
4.11	Intestinal morphology of broilers fed on diets supplemented with different rates of <i>A. paniculata</i> and <i>O. stamineus</i> ground leaf (Mean $\pm$ SE).	65
5.1	Ingredients in the basal diet and nutritional analysis.	75
5.2	Live body weight and weight gain of broilers fed different diets for three weeks.	82
5.3	Feed intake, FCR and mortality of broilers fed different diets for three weeks.	83
5.4	Effect of experimental diets on carcass yield and relative Organ/tissue weights of broilers on day-42.	84
5.5	Serum biochemical parameters and experimental outcomes of broilers on day-42.	85
5.6	Lipid peroxidation in serum and liver of broilers on day-42.	86
5.7	Intestinal bacteria population (log CFU/g of intestinal contents) of broiler chickens fed different diets (Readings on Day-42).	87
5.8	Water holding capacity (WHC), total phenolic content and proximate analysis of the breast meat of broiler chickens on day-42.	88
5.9	Sensory scores of cooked breast meat of broiler chickens fed on different diets.	89
5.10	Colour changes of raw breast meat broiler chickens subjected to different dietary treatments in relation to duration of storage at 4°C.	90
5.11	Changes in antioxidative potential of breast meat of broiler chickens in relation to duration of storage at 4°C.	92

## LIST OF FIGURES

Figure		Page
2.1	<i>Andrographis paniculata</i> Nees, common name associated with this medicinal plant including, King of Bitter, and several other names from Malay Archipelago such as <i>Hempedu Bumi</i> , <i>Pokok Cerita</i> , <i>Pasak Bumi</i> and <i>Setunjang Bumi</i> .	14
2.2	<i>Orthosiphon stamineus</i> Benth, common names associated with this medicinal plant including, Cat Whiskers and Kidney Tea Plant, and several other names from Malay Archipelago such as <i>Misai Kucing</i> , <i>Kumis Kucing</i> , <i>Remujung</i> , <i>Ruku Hitam</i> , and <i>Teh Jawa</i> (Java Tea).	17
3.1	HPLC chromatograms (absorbance at 230 nm) of andrographolide (AP) from <i>Andrographis paniculata</i> leaves extracted with different solvents: (a) reference marker; (b) water extract; (c) ethanol extract; (d) methanol extract; (e) chloroform extract.	29
3.2	HPLC chromatograms (absorbance at 340 nm) of rosmarinic acid (RA) from <i>Orthosiphon stamineus</i> leaves extracted with different solvents: (a) reference marker; (b) water extract; (c) ethanol extract; (d) methanol extract; (e) chloroform extract (the arrow indicates the RA was undetected).	32
3.3	Susceptibility of pathogenic bacteria to <i>Andrographis paniculata</i> and <i>Orthosiphon stamineus</i> extracts prepared with different solvents. Note: Ctrl: Control; Ac: Acetic acid (10 %); Tc: Tetracycline (30 µg/disc). Heights of colored bars represent inhibition diameters.	34
3.4	Total phenol contents of <i>Andrographis paniculata</i> and <i>Orthosiphon stamineus</i> leaf extracts prepared with different solvents. Values in each column bearing the same letter are not significantly different (P>0.05).	35
3.5	DPPH scavenging activity of <i>A. paniculata</i> and <i>O. stamineus</i> extracts prepared with different solvents. Values in each column bearing the same letter are not significantly different (P>0.05).	36
3.6	Superoxide scavenging activity of <i>A. paniculata</i> and <i>O. stamineus</i> extracts prepared with different solvents. Values in each column bearing the same letter are not significantly different (P>0.05).	37

- 4.1 Histological examination on liver of 42-day old broilers fed control diet, Diet AP2, Diet AP4, Diet AP8, Diet OS2, Diet OS4, and Diet OS8. All figures show normal liver histology where hepatocytes (H), sinusoid (S), central vein (CV) and portal area conditions were at normal (40X magnification). 67
- 5.1 The pH of raw breast meat of broiler chickens on different diets. Changes over duration of storage at 4°C. 91



## LIST OF ABBREVIATIONS

ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
AME	Apparent metabolizable energy
AP	<i>Andrographis paniculata</i>
AST	Aspartate Aminotransferase
CFU	Colony Forming Unit
CP	Crude Protein
DM	Dry Matter
EU	European Union
FAO	Food and Agriculture Organization
FCR	Food Conversion Ratio
FDA	Food and Drug Association
GE	Gross Energy
GAE	Gallic Acid Equivalent
HPLC	High Performance Liquid Chromatography
IC <sub>50</sub>	Inhibitory Concentration at 50 %
NRC	National Research Council
OS	<i>Orthosiphon stamineus</i>
T20	Tetracycline
USDA	United States Department of Agriculture
VE	Vitamin E

## Units

°C	degrees centigrade
cm	centimeter
g	gram
g/day	gram per day
g/kg	gram per kilogram
h	hour
l	liter
kg	kilogram
MJ/kg	megajoule per kilogram
min	minute
mbar	millibar
mg	milligram
ml	milliliter
mm	millimeter
mmol/L	millimol per liter
ml/min	milliliter per minute
mM	millimol
mg/ml	milligram per milliliter
M	molar
μm	micrometer
μL	microliter
μg	microgram
ppm	parts per million
sec	second

### **Common abbreviations**

et al.	and others
%	percentage
e.g	for example

### **Statistical terms**

ANOVA	Analysis of variance
CRD	Completely Randomized Design
SE	Standard Error

### **Chemical elements and compounds**

ABTS	2,2- azinobis- (3 ethylbenzothiazoline-6-sulfonic acid)
ACN	acetonitrile
DPPH	2, 2- diphenyl -1-picrylhydrazyl
HCl	Hydrochloric acid
H <sub>2</sub> SO <sub>4</sub>	Sulphuric acid
MHA	Muller Hinton Agar
RA	Rogosa Agar



## CHAPTER 1

### GENERAL INTRODUCTION

Broiler chicken represents 29% of meat production from farmed animals and this value rising each year. Poultry meat and eggs offer considerable potential for meeting human needs for dietary animal protein supply. With regards to the growth of broiler industry, Malaysia ranked seventh place in the world (Anon, 2012). In Malaysia, the broiler industry is expected to grow at the rate of 4% in 2013, outpacing global production growth at 2.5% (Lim, 2013).

To improve broiler production and satisfy market demand, it has been the common practice for farmers to use synthetic antibiotics and antioxidant in the feed as growth promoters. Antibiotics including chlortetracycline, tetracycline, virginiamycin, spiramycin, tylosin phosphate, zinc bacitracin and avopracin as growth promoters have been used for decades in poultry production for improving farm performance and controlling diseases (Huyghebaert *et al.*, 2011). With increasing interests in discontinuing the use of antibiotics due to their harmful effects to environment, actively efforts to search for safe, suitable and viable alternatives to the antibiotic growth promoters has become intensified. Ideally, these alternative growth promoters should improve growth performance, as do the antibiotics, and maintain a sound health of the chickens. Besides the need of healthy grown broilers, meat quality also needs to give much attention. Oxidative stability is a central parameter in the estimation of meat quality because of the susceptibility of this food product to oxidative degeneration, which is one of the main causes of spoilage (Morrissey *et al.*, 1998). The shelf life of meat is related to lipid oxidation reactions which could affect its sensory properties, by causing rancidity, as well as its nutritional characteristics through the formation of potentially toxic compound (Morrissey and Kerry, 2004). Conventionally, oxidation of chicken meat can be prevented by synthetic antioxidants including butylated hydroxyanisole (BHA), butylated hydroxy toluene (BHT) and propyl gallate (PG) but their safety has been questioned (Barlow, 1990).

Moreover, the excessive use of these synthetic antibiotics and antioxidants has led to contamination of broiler meat and environment. For example, the practice of feeding antibiotics to livestock leads to antibiotic-resistant bacteria that are dangerous to human health. Frequently and excessively used of antibiotics cause harmful bacteria become resistant to the drug, and the treatment becomes less effective (Huyghebaert *et al.*, 2011). In fact, bacteria can develop into a completely different strain that cannot be killed by the normally prescribed antibiotic. Synthetic antioxidants have been restricted recently, mainly because of their possible carcinogenicity causing liver swelling and changing liver enzyme activities. With the increasing consciousness of public consumers on safety of food additive, therefore, an urgent need for identifying alternative natural and probably safer sources of antibiotics and antioxidants.

Currently, the interest in natural antioxidants and antibiotics have increased dramatically because they are considered to be safer than the synthetics, and have greater application potential for consumers acceptability, palatability, stability and shelf-life of meat products (Kang *et al.*, 2008). The medicinal herbs have been used since ancient times not only for flavouring foods but also for their remedies. The preservative effect of spices and herbs suggests the presence of antimicrobial and antioxidative constituents (Basmacioglu *et al.*, 2004; Al-Marzooqi *et al.*, 2010).

In the present study, two species of medicinal plants have been selected namely, *Andrographis paniculata* Nees, (Acanthaceae) and *Orthosiphon stamineus* Benth, (Lamiaceae), they are common in Southeast Asia, India and China. *A. paniculata* has been traditionally used as an antioxidant, antiviral, anti-inflammatory, immune enhancing agent and hepatoprotective (Prajjal *et al.*, 2003). Its active component has been reported to have anti-cancer (Sheeja and Kuttan, 2007), anti-HIV (Calabrese *et al.*, 2000), and antimicrobial (Roy *et al.*, 2010) properties. The other herb, *Orthosiphon stamineus*, has been used to treat urinary lithiasis, edema, eruptive fever, influenza, rheumatism, hepatitis, jaundice and biliary lithiasis (Akowuah *et al.*, 2005). *O. stamineus* leaf is consumed as Java tea to facilitate body detoxification (Chin *et al.*, 2008). Both plants have been reported containing natural antibiotics and antioxidants (Prajjal *et al.*, 2003; Ho *et al.*, 2010). However, very few information of medicinal plants as dietary supplements in animals, especially *Andrographis paniculata* and *Orthosiphon stamineus*. The hypotheses of the present study were:

1. *Andrographis paniculata* and *Orthosiphon stamineus* are species of medicinal plants containing antibiotic and antioxidant compounds.
2. Supplementation of *A. paniculata* and *O. stamineus* ground leaf in the diets improve growth and meat quality in broiler chickens.

The goal of this research work was to evaluate the possibilities of improving broiler chicken performance using *A. paniculata* and *O. stamineus* leaf preparation as feed supplement as opposed to the synthetic antibiotics and antioxidant. The specific objectives set were:

1. To determine the antibiotic and antioxidant properties of *A. paniculata* and *O. stamineus* leaf extracts in different solvents, and to detect and quantify andrographolide and rosmarinic acid presence in the respective species.
2. To evaluate the effect of different levels of *A. paniculata* and *O. stamineus* ground leaf supplemented in diets on growth performance, carcass characteristics and blood biochemistry of broiler chickens, and also to determine the morphological and histological changes in gastrointestinal tract and in the liver.
3. To evaluate the antibiotic and antioxidant potential of *Orthosiphon stamineus* ground leaf on growth performance, meat characteristics, intestinal microflora and lipid peroxidation of broiler chickens.

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